A Study on Evaluation of Analgesic Potential of Newly Synthesized Compounds

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Abstract- **While conventional pain management methods are losing their efficacy, a growing body of pharmacological research suggests that natural products may be useful in the creation of novel compounds or treatments. Moreover, recently, there has been an increasing interest to find new and analgesic drugs with possibly fewer side effects from natural sources and medicinal plants. Hence, we aimed to evaluate the analgesic activities of newly synthesized ligands from natural sources. The measurement of analgesics activity of newly synthesized ligands was performed using hot plate method in experimental animal model study. A total of 28 female Sprague-Dawley rats were equally divided in to seven group (n=4) *viz.* Group 1 to Group VII. Group I and Group II served as normal control and positive control group wherein animals received normal saline (10ml/kg; orally) and Buprenorphine (0.05mg/kg; i.p.) respectively. Animals in Group III, IV, V, VI, and VII received ligands L3 or L4, L3 or L4 + Zn, L3 or L4 + Ni, L3 or L4 + Cu, L3 or L4 + Co respectively. Results revealed that animals treated with ligands either L3 or L4 compounds exhibited significant analgesic activities ($p<0.05$). Furthermore, the results indicates that tested compound L3 and L4 both works similar to well-established drug Buprenorphine. Both L3 and L4 exhibited moderate analgesic activity at 30 mins and activity was increased at 60min and reached the maximum peak at 180min. In conclusion, significant analgesic activities of newly synthesized ligands L3 and L4 was observed, and hence these ligands could be explored for the development of natural analgesic drugs.**

Keywords: **Ligands, Analgesic, Pain, Natural products, Opioids, Buprenorphine.**

I. INTRODUCTION

At the same time, as conventional therapies for the treatment of pain are losing their effectiveness, more and more pharmacological studies are showing that products of natural origin are promising for the development of new molecules or therapies.[1] According to the International Association for the Study of Pain (IASP) pain is defined as "an unpleasant sensory and actual or perceived injury to body tissues and produce physical and emotional reaction".[2] Pain arising from the skin and from the deep structure like muscle, bones and joints is termed as somatic pain. It is usually well defined and is generally caused by inflammatory reaction in the tissue, and it may be accompanied by contraction of the surrounding skeletal muscle as in patient with rheumatoid arthritis.[3,4] Pain is a crucial aspect of the body's defense mechanisms, and it is a part of a rapid warning relay instruction the motor neurons of the central nervous system to minimize physical harm.[5] Pain can be classified into two types *viz.* acute pain and chronic pain.

Pain is in generally seen as nociceptive,[6] inflammatory or neuropathic response.[7] Pain is common nonspecific manifestations of many diseases. Thousands of patients with intense pain, such as that resulting from cancer or severe injury, must depend on current regimens (peripheral or centrally acting) like morphine, aspirin and non-steroidal anti-inflammatory drugs.[8,9] Although non-steroidal anti-inflammatory drugs (NSAIDs) and opiates have been used classically in these conditions, but some adverse reactions occur with these drugs such as gastrointestinal disturbances, renal damage, respiratory disorders, depression and possible dependence.[10]

In recent years, there has been an increasing interest to find new anti-inflammatory and analgesic drugs with possibly fewer side effects from natural sources and medicinal plants. Opioids play an essential role in pain management especially for moderate to severe pain. They are used for acute pain, in palliative care and pain treatment of degenerative conditions, morphine is the

prototype opioid. Antidepressants and anti-epileptics are also used in pain management.[11] In this context, we synthesized new ligands and explored the biological activity, analgesic potential of newly synthesized ligands from natural sources.

II. MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats (150-200 g) were obtained from the central animal house of H. S. K. College of Pharmacy and Research Centre, Bagalkot, Karnataka. All animals were kept under standard husbandry conditions (Temp. 22-28^{0}C; Relative Humidity 65±10%) for 12hr dark and 12hr light cycle respectively in standard propylene cages. The animals were fed with standard food (Pranav Agro Industries, Sangli, Maharashtra) and water *ad libitum*. All the experiments were conducted in accordance with direction of Institutional Animals Ethics Committee.

Acute toxicity study

The female Sprague-Dawley rats (150-200 g) were used and acute toxicity was studied according to the OECD guidelines 425. Limit dose of 2000 mg/kg (p.o.) was administered and they were observed for behavior and other signs of toxicity, such as excitation, tremors, twitches, motor coordination, righting reflex and respiratory changes for 4hrs and monitored up to 14 days. No mortality was occurred with higher limit dose.

Analgesics activity

The measurement of analgesics activity of test samples was performed using hot plate method in experimental animal model study. This experiment was performed using over-night starved female Sprague-Dawley rats (150-200 g) rats. Animals were divided into 7 groups with each group containing 4 animals as follows:

Group I: Control group received the normal saline (10ml/kg) orally (n=4)
Group II: Positive control group received Buprenorphine (0.05mg/kg; i.p.) (n=4)
Group III: Animals received L (Ligand 3 or Ligand 4) sample, (n=4)
Group IV: Animals received L (L3 or L4) +Zn sample, (n=4)
Group V: Animals received L (L3 or L4) +Ni sample, (n=4)
Group VI: Animals received L (L3 or L4) +Cu sample, (n=4)
Group VII: Animals were received L (L3 or L4) +Co sample, (n=4)

The Eddy and Leimbach hot plate experiment was carried out as follows. Animal groups were administered with either 0.8% NaCl for control group (Group I) or Buprenorphine (0.05mg/kg) for positive control group or test compounds (Group III to group VII: L (L3 or L4), L (L3 or L4) + Zn, L (L3 or L4) + Ni , L3 (L3 or L4) + Cu, L(L3 or L4) + Co respectively) as an aqueous suspension prepared in Tween 80 (10% v/v). Following the administration, at different time points (60 min, 120 mins and 180 mins after administration) rats were kept on hot plate pre heated to 55 ± 0.5°C for 15sec. Response time of animals were noted when the rats jumped or licked its hind paw. The cut off time is fixed for 15sec to prevent injury to paw. The time taken to lick the hind paw was recorded at 0min, 60min 120min and 180min. Increase in reaction time (time interval taken by animal to lick paw) were considered as proportional to analgesic activity.

III. RESULTS

The results of the analgesic effect of the control, Buprenorphine and test samples (L3 or L4 and its metal derivatives) using hot plate method are presented in Table 1 and 2. When comparted to control group, animals treated with the standard drug Buprenorphine (0.05mg/kg) showed significant analgesic activity (p<0.001) after 60 min, 120 min and 180 min of exposer. Animal group exposed for longer time (180 min) took more time to exhibit response. Animal group III treated with either L3 or L4 compound also exhibited significant response (p<0.05) and response time was similar to that of standard drug Buprenorphine, indicating that L3 and L4 compounds have analgesic activity. Analgesic activity of metal derivatives of L3 and L4 (L3 or L4 + Zn, L3 or L4 + Ni, L3 or L4 + Cu and L3 or L4 + Co) were similar to that of L3/L4 compound indicating that linking of metal (Zn, Ni, Cu, Co) to L3 and l4 compound has no impact on analgesic activity.

Table 1: Effect of L3 and its derivatives on analgesic activity by Eddy and Leimbach hot plate method

Groups	Response Time (in seconds)			
	0 min after exposure	60 min after exposure	120 min after exposure	180 min after exposure
Control	3.075±0.047	3.050±0.028	3.000±0.040	2.900±0.040
Buprenorphine	3.025±0.047***	3.975±0.047***	5.300±0.040***	6.175±0.047***
L3	3.050±0.028***	4.200±0.040***	5.000±0.070***	6.050±0.064***
L3 + Zn	3.075±0.047***	4.200±0.040***	5.075±0.047***	6.225±0.047***
L3 + Ni	3.100±0.040***	3.950±0.288***	5.200±0.040***	6.150±0.064***
L3 + Cu	2.950±0.028***	3.975±0.047***	5.125±0.478***	6.025±0.047***
L3 + Co	2.975±0.047***	4.225±0.062***	5.325±0.062***	6.200±0.040***

L3-Ligand
Values are expressed as mean ± SEM, n=4,
***p<0.001 as compared to control group based on One way ANOVA followed by Dunnett's multiple comparison *post-hoc* test

Table 2: Effect of L4 and its derivatives on analgesic activity by Eddy and Leimbach hot plate method

Groups	Response Time (in seconds)			
	0 min after exposure	60 min after exposure	120 min after exposure	180 min after exposure
Control	3.075±0.047	3.050±0.028	3.000±0.040	2.900±0.040
Buprenorphine	3.025±0.047***	3.975±0.043***	5.300±0.040***	6.175±0.047***
L4	3.100±0.024***	4.250±0.036***	5.050±0.050***	6.100±0.060***
L4 + Zn	3.125±0.043***	4.250±0.038***	5.125±0.043***	6.275±0.045***
L4 + Ni	3.150±0.036***	4.000±0.284***	5.250±0.038***	6.200±0.062***
L4 + Cu	3.000±0.024***	4.025±0.043***	5.175±0.474***	6.075±0.045***
L4 + Co	3.025±0.043***	4.275±0.060***	5.375±0.060***	6.250±0.042***

L4-Ligand
Values are expressed as mean ± SEM, n=4,
***p<0.001 as compared to control group based on One way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparisons *post-hoc* test

IV. DISCUSSION

Today, drug discovery has become a complex field far beyond the use of only natural products. However, natural products have dominated the drug industry for many years and several marketed drugs are based on isolates from such. There has been a recent resurgence in the study of natural products, especially from the dietary supplement industry. The pharmaceutical industry has begun to revitalize programs on the screening of natural products. Substances derived from natural products have been utilized since the beginning of time for various purposes including the treatment of pain. Opium, for example, has been used since the earliest records of time, some 7000 years ago.[12] Severe pain is usually relieved with opioid analgesics with high intrinsic activity whereas sharp, intermittent pain does not appear to be as effectively controlled. The pain associated with cancer and other terminal illnesses must be treated aggressively and often requires a multidisciplinary approach for effective management. Since existing drugs exhibits adverse effect on biological activity of patients, there is renewed interest in new class of analgesics.[8] Hence, in the current study we aimed to evaluate the analgesic activities of newly synthesized ligands from natural sources.

Results of our study delineated that animals treated with ligands either L3 or L4 compounds exhibited significant analgesic activities ($p<0.05$). Furthermore, the results indicates that tested compound L3 and L4 both works similar to well-established drug Buprenorphine. Both L3 and L4 exhibited moderate analgesic activity at 30 mins and activity was increased at 60min and reached the maximum peak at 180min. These findings could be validated through

analgesic activities exhibited in various medicinal plant extracts reported in the literature.

Ilmi et al., observed *Andrographis paniculata* ethyl acetate fraction exhibited analgesic activities in the tablet dosage form indicating that this species could be an excellent candidate as an herbal medicine for the treatment of pain.[13] Liao et al., studied the effects of *Scutellaria baicalensis*, a traditional Chinese medicine used for the treatment of inflammatory and painful conditions, on migraine by behavioral analysis of systemic administration to rats using the nitroglycerin induced migraine rat model. Authors observed that pretreatment with 1.0 g/kg *Scutellaria baicalensis* alleviated migraine-related behaviors in the nitroglycerin -induced experimental model. Thus, *Scutellaria baicalensis* may be a promising natural product for the treatment of migraine.[14]

In another research investigation carried out by Chang et al., investigated the molecular mechanism as well as the effective compounds present in the Gu-tong formula. Gu-tong formula is used in treatment of cancer-related pain and includes nine traditional Chinese medicines. Authors revealed the potential pharmacological mechanism of Gu-tong formula in cancer pain treatment, from a systematic perspective, which may involve the secretion of inflammatory cytokines, membrane potential, bone protection, and other biological processes through the regulation of chemokines, MAPK, and TRP channels. Cholesterol and stigmasterol in Gu-tong formula have been suggested to be the key pharmacodynamic molecules for analgesia, as seen in molecular docking screening. These findings provide insights into comprehending the synergistic effect of Gu-tong formula on cancer pain relief.[15]

It is very evident that natural products have been and continue to be a valuable source of novel compounds and peptides that have the potential to serve as analgesic agents or as lead molecules for the development of such agents. As more research is conducted on natural products it is inevitable that more diverse compounds will be discovered and new mechanisms of action will be elucidated. There is much reason for excitement for the future of natural products research, particularly with regard to the development of novel agents that interact with nociceptive pathways. The fields of pharmacognosy and medicinal chemistry will work closely to ensure that novel compounds of natural origin are explored for their potential in the development of novel drug candidates.[12]

V. CONCLUSION

In conclusion, analgesic drugs which are currently in use are either narcotics or nonnarcotics which have proven side and toxic effects. To develop new synthetic compounds in this category is an expensive venture and again may have problems of side effects. On the contrary, many medicines of plant origin had been used and are in use successfully since long time without any serious effects. The results of this preliminary pilot study demonstrated the significant analgesic activities of newly synthesized ligands L3 and L4, and hence these ligands could be explored for the development of natural analgesic drugs.

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